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A new species of the genus *Leptobrachella* Smith, 1935 (Anura, Megophryidae) from northwestern Guangxi, China

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Abstract

In this study, we describe a new species of the species-rich genus *Leptobrachella*, *Leptobrachella yachangensis* **sp. nov.**, from northwestern Guangxi, China, based on a combination of molecular and morphological data. The new species is a sister taxon to *L. wumingensis* Chen, Peng, Li & Yu, 2023, with discrete, diagnostic differences from all other congeners. We also discussed the phenomenon and causes of the high diversity within this genus as well as the conservation of its congeners.

Key Words

conservation, diversity, morphology, molecular phylogeny, taxonomy

Introduction

The genus *Leptobrachella* Smith, 1925 represents one of the most species-rich groups of frogs with 110 recognized species (Frost 2025). Congeners of this genus are forest dwellers that are widely distributed in China, Myanmar,northeastern India, the Indochina region, Borneo and NatunaIsland (Frost 2025). The morphological conservativeness hinders our understanding of the true diversity of this group (Wang et al. 2019; Wang et al. 2022).

During recent field surveys in mountainous areas of northwestern Guangxi (Fig. 1), we collected seven adult male and two adult female specimens of a *Leptobrachella* species. Preliminary morphological examination indicated that they can be distinguished from recognized congeners by a series of discrete features. Subsequent molecular analysis further revealed that these specimens represent a separate evolutionary lineage. Considering both the morphological differences and their genetic allocation and divergences, these specimens are described herein as a new species.

Materials and methods

Sampling

During our field work in northwestern Guangxi, three *Leptobrachella* individuals were collected from Yachang Orchid National Nature Reserve. All newly collected specimens were euthanized with MS-222, fixed in 10% buffered formalin, later transferred to 70% ethanol for long-term preservation, and deposited at the Natural History

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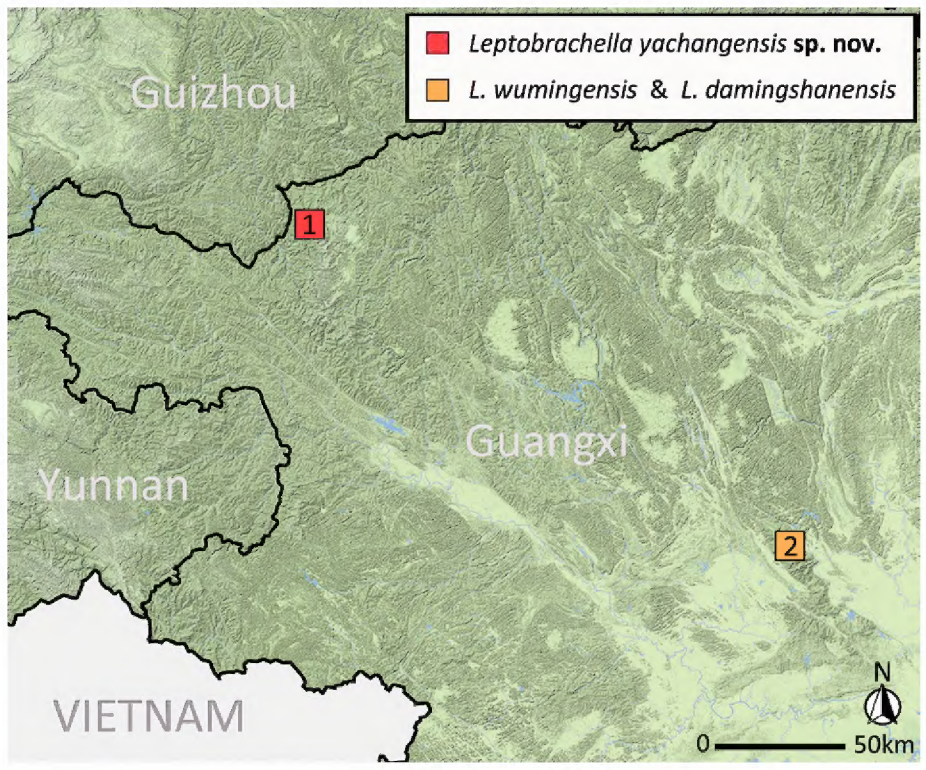

Figure 1. Distribution sites of *Leptobrechella yachangensis* sp. nov., *L. damingshanensis* and *L. wumingensis*.

Museum of Guangxi (NHMG), Nanning City, Guangxi, China. Muscle tissue samples collected from thigh were preserved in 95% ethanol for molecular analysis.

Phylogenetic analyses

DNA extraction, PCR, and sequencing for the three newly collected samples followed Wang et al. (2020). The 16S ribosomal RNA mitochondrial gene (16S rRNA) fragment of 509 base pairs was sequenced for new samples. In total, 74 samples of 64 *Leptobrachella* species from China along with two outgroup samples were used in this study, encompassing three newly sequenced individuals and others downloaded from GenBank. Detailed information for all samples is given in Suppl. material 1: table S1.

Sequences were aligned using Clustal X 2.0 (Thompson et al. 1997) with defaultparameters. We ran Jmodeltest v2.1.2 (Darriba et al. 2012; with Akaike and Bayesian information criteria) on our alignment and obtained the best-fitting nucleotide substitution model of GTR + I + G (General Time Reversible model, with variable sites modelled according to the Gamma distribution, and a portion of the sites invariant). Phylogenetic analysis was conducted using Bayesian inference (BI) in MrBayes 3.2.4 (Ronquist et al. 2012) and maximum likelihood (ML) in maximum likelihood (ML) in Randomized Axelerated Maximum Likelihood (RAxML, Stamatakis 2006) with RAxML GUI 1.3 (Silvestro and Michalak 2012). For the ML analysis, an optimal tree was obtained and branch supports were evaluated with 1000 rapid bootstrapping replicates. For the BI analysis, two independent runs with four Markov Chain Monte Carlo simulations were performed for ten million iterations and sampled every 1000 iterations. The first 25% of the samples were discarded as burn-in, leaving 7500 samples in the final summary. Convergence of the Markov Chain Monte Carlo simulations was assessed by PSRF < 0.01 and ESS (effective sample size) value > 200 using Tracer 1.4 (http://tree.bio.ed.ac.uk/software/tracer/). Nodes having ML bootstrap values (BS) ≥ 70 and BI posterior probabilities (BPP) ≥ 0.90 were considered well supported. Genetic distances among all *Leptobrachella* samples were calculated in MEGA 11 using the uncorrected *p*-distance model with complete deletion of for gaps ormissing data.

Morphological examination

Following Fei et al. (2009) and Rowley et al. (2013), measurements were taken with digital calipers from preserved specimens (Neiko 01407A Stainless Steel 6-Inch Digital Calipers) to the nearest 0.1 mm. Measurements include: snout-vent length (SVL) from the tip of the snout to posterior margin of the vent; head length (HDL) from the tip of the snout to the articulation of the jaw; head width (HDW) at the commissure of the jaws; snout length (SNT) from the tip of the snout to the anterior corner of the eye; eye diameter (EYE) from the anterior corner of the eye to posterior corner of the eye; internasal distance from inner margins of nostrils (IND); interorbital distance (IOD); horizontal diameter of tympanum (TMP); tympanum-eye distance (TEY) from the anterior edge of tympanum to posterior corner of the eye; tibial length (TIB) from the outer surface of the flexed knee to the heel; manus length (ML) from the tip of the third finger to the proximal edge of inner palmar tubercle; pes length (PE) from the tip of the fourth toe to the proximal edge of the inner metatarsal tubercle; length of lower arm and hand (LAHL) from the tip of the third finger to elbow; and hindlimb length (HLL) from the tip of the fourth toe to vent.

Sex was determined by the presence of vocal sac (and vocal sac openings) in males (vs. absence in females). Comparative morphological data for other congeneric species of *Leptobrachella* were obtained from the literature (Table 1).

Results

The ML and Bayesian analysis both resulted identical topologies and strongly support that all *Leptobrachella* samples formed a monophyletic clade (Fig. 2). In the phylogenetic topology, the *Leptobrachella* samples from Yachang Orchid National Nature Reserve form a monophyletic lineage with strong support (BS = 100; BPP = 1.00). Though with low support, both ML and Bayesian analysis result that the new lineage is a sister taxon to *L. wumingensis*. Besides, the new lineage from Yachang Orchid National Nature Reserve obviously differs from its closely-related congeners by mean *p*-distance ≥ 6.5% in the 16S RNA ribosomal mitochondrial gene, which significantly exceeds the level of genetic divergence observed in recognized species of *Leptobrachella*.

Our detailed morphological examination has also revealed discrete, diagnostic (non-overlapping ranges in traditional characters) differences among the specimens from this independent lineage and all other congeners. Therefore, both the phylogenetic result and morphological comparison support the hypothesis that the lineage from Yachang Orchid National Nature Reserve represent an undescribed new species, and herein we describe this new species.

Table 1. Data source of the currently known species of the genus *Leptobrachella*.

ID	*Leptobrachella* species	Literature obtained
1	*L. aerea* (Rowley, Stuart, Richards, Phimmachak & Sivongxay, 2010c)	Rowley et al. 2010c
2	*L. albomarginata*Wu, Yu, Kilunda, Murphy & Che, 2025	Wu et al. 2025
3	*L. aspera* Wang, Lyu, Qi & Wang, 2020	Wang et al. 2020
4	*L. alpina* (Fei, Ye & Li, 1990)	Fei et al. 1990; Fei et al. 2009, 2016
5	*L. applebyi* (Rowley & Cao, 2009)	Rowley and Cao 2009
6	*L. arayai* (Matsui, 1997)	Matsui 1997
7	*L. ardens* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
8	*L. aurantirosea* Ninh, Nguyen, Le, Nguyen, Quoc, Orlov, Bezman-Moseyko, Le, Nguyen & Ziegler, 2024	Ninh et al. 2024
9	*L. batxatensis* Hoang, Pham, Phan, Do, Wang, Jiang & Nguyen, 2025	Hoang et al. (2025)
10	*L. baluensis* Smith, 1931	Dring 1983; Eto et al. 2016
11	*L. bashaensis* Lyu, Dai, Wei, He, Yuan, Shi, Zhou, Ran, Kuang, Guo, Wei & Yuan, 2020	Lyu et al. 2020a
12	*L. bijie* Wang, Li, Li, Chen & Wang, 2019	Wang et al. 2019, 2022; Wang et al. 2021
13	*L. bidoupensis* (Rowley, Le, Tran & Hoang, 2011)	Rowley et al. 2011
14	*L. bondangensis* Eto, Matsui, Hamidy, Munir & Iskandar, 2018	Eto et al. 2018
15	*L. botsfordi* (Rowley, Dau & Nguyen, 2013)	Rowley et al. 2013
16	*L. bourreti* (Dubois, 1983)	Ohler et al. 2011
17	*L. brevicrus* Dring, 1983	Dring 1983; Eto et al. 2015
18	*L. crocea* (Rowley, Hoang, Le, Dau & Cao, 2010)	Rowley et al. 2010a
19	*L. chishuiensis* Li, Liu, Wei & Wang, 2020	Li et al. 2020
20	*L. damingshanensis* Chen, Yu, Cheng, Meng, Wei, Zhou & Lu, 2021	Chen et al. 2021b
21	*L. dayaoshanensis* Chen, Yu, Meng & Qin, 2024	Chen et al. 2024
22	*L. dong* Liu, Shi, Li, Zhang, Xiang, Wei & Wang, 2023	Liu et al. 2023
23	*L. dorsospina* Wang, Lyu, Qi & Wang, 2020	Wang et al. 2020
24	*L. dringi* (Dubois, 1987)	Inger et al. *1*995; Matsui and Dehling2012
25	*L.dushanensis* Li, Li, Cheng, Liu, Wei, Wang, 2024	Li et al. 2024
26	*L. duyenae* Hoang, Pham, Phan, Do, Wang, Jiang & Nguyen, 2025	Hoang et al. 2025
27	*L. eos* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011)	Ohler et al. 2011
28	*L. feii* Chen, Yuan & Che, 2020	Chen et al. 2020
29	*L. firthi* (Rowley, Hoang, Dau, Le & Cao, 2012)	Rowley et al. 2012
30	*L. fritinniens* (Dehling & Matsui, 2013)	Dehling and Matsui 2013
31	*L. fuliginosa* (Matsui, 2006)	Matsui 2006
32	*L. flaviglandulosa* Chen, Wang & Che, 2020	Chen et al. 2020
33	*L. fusca* Eto, Matsui, Hamidy, Munir & Iskandar, 2018	Eto et al. 2018
34	*L. gracilis* (Günther, 1872)	Günther 1872; Dehling 2012b
35	*L. graminicola* Nguyen, Tapley, Nguyen, Luong & Rowley, 2021	Nguyen et al. 2021
36	*L. guinanensis* Chen, Li, Peng & Liu, 2024	Chen et al. 2024
37	*L. hamidi* (Matsui, 1997)	Matsui 1997
38	*L. heteropus* (Boulenger, 1900)	Boulenger 1900
39	*L. huynhi* Hoang, Luong, Nguyen, Nguyen, Ninh, Le, Ziegler & Pham, 2024	Hoang et al. 2024
40	*L. isos* (Rowley, Stuart, Neang, Hoang, Dau, Nguyen & Emmett, 2015)	Rowley et al. 2015a
41	*L. itiokai* Eto, Matsui & Nishikawa, 2016	Eto et al. 2016
42	*L. juliandringi* Eto, Matsui & Nishikawa, 2015	Eto et al. 2015
43	*L. jinshaensis* Cheng, Shi, Li, Liu, Li & Wang, 2021	Cheng et al. 2021
44	*L. jinyunensis* Shi, Shen, Wang, Jiang & Wang, 2023	Shi et al. 2023
45	*L. kajangensis* (Grismer, Grismer & Youmans, 2004)	Grismer et al. 2004
46	*L. kalonensis* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
47	*L. kecil* (Matsui, Belabut, Ahmad & Yong, 2009)	Matsui et al. 2009
48	*L. khasiorum* (Das, Tron, Rangad & Hooroo, 2010)	Das et al. 2010
49	*L. korifi* Matsui, Panha & Eto, 2023	Matsui et al. 2023
50	*L. lateralis* (Anderson, 1871)	Anderson 1871; Humtsoe et al. 2008
51	*L. laui* (Sung, Yang & Wang, 2014)	Sung et al. 2014
52	*L. liui* (Fei & Ye, 1990)	Fei et al. 1990; Fei et al. 2009; Sung et al. 2014; Wang et al. 2022
53	*L. macrops* (Duong, Do, Ngo, Nguyen & Poyarkov, 2018)	Duong et al. 2018
54	*L. maculosa* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
55	*L. mangshanensis* (Hou, Zhang, Hu, Li, Shi, Chen, Mo & Wang, 2018)	Hou et al. 2018; Wang et al. 2022
56	*L. maoershanensis* (Yuan, Sun, Chen, Rowley & Che, 2017)	Yuan et al. 2017
57	*L. marmorata* (Matsui, Zainudin & Nishikawa, 2014b)	Matsui et al. 2014b
58	*L. maura* (Inger, Lakim, Biun & Yambun, 1997)	Inger et al. 1997
59	*L. melanoleuca* (Matsui, 2006)	Matsui 2006
60	*L. melica* (Rowley, Stuart, Neang & Emmett, 2010)	Rowley et al. 2010b
61	*L. minima* (Taylor, 1962)	Taylor 1962; Ohler et al. 2011
62	*L. mjobergi* Smith, 1925	Eto et al. 2015
63	*L. murphyi* Chen, Suwannapoom, Wu, Poyarkov, Xu, Pawangkhanant & Che, 2021	Chen et al. 2021a

ID	*Leptobrachella* species	Literature obtained
64	*L. nahangensis* (Lathrop, Murphy, Orlov & Ho, 1998)	Lathrop et al. 1998
65	*L. natunae* (Günther, 1895)	Günther 1895
66	*L. namdongensis* Hoang, Nguyen, Luu, Nguyen & Jiang, 2019	Hoang et al. 2019
67	*L. neangi* Stuart & Rowley, 2020	Stuart and Rowley 2020
68	*L. niveimontis* Chen, Poyarkov, Yuan & Che, 2020	Chen et al. 2020
69	*L. nokrekensis* (Mathew & Sen, 2010)	Mathew and Sen 2010
70	*L. nyx* (Ohler, Wollenberg, Grosjean, Hendrix, Vences, Ziegler & Dubois, 2011)	Ohler et al. 2011
71	*L. oshanensis* (Liu, 1950)	Liu, 1950; Shi et al. 2021
72	*L. pallida* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
73	*L. palmata* Inger & Stuebing, 1992	Inger and Stuebing 1992
74	*L. parva* Dring, 1983	Dring 1983
75	*L. pelodytoides* (Boulenger, 1893)	Boulenger 1893; Ohler et al. 2011
76	*L. petrops* (Rowley, Dau, Hoang, Le, Cutajar & Nguyen, 2017)	Rowley et al. 2017a
77	*L. phiadenensis* Luong, Hoang, Pham, Ziegler & Nguyen, 2023	Luong et al. 2023
78	*L. phiaoacensis* Luong, Hoang, Pham, Ziegler & Nguyen, 2023	Luong et al. 2023
79	*L. pingbianensis* (Rao, Hui, Zhu & Ma, 2022 "2020")	Rao et al. 2022 "2020"
80	*L. picta* (Malkmus, 1992)	Malkmus 1992
81	*L. platycephala* (Dehling, 2012)	Dehling 2012a
82	*L. pluvialis* (Ohler, Marquis, Swan & Grosjean, 2000)	Ohler et al. 2000, 2011
83	*L. puhoatensis* (Rowley, Dau & Cao, 2017)	Rowley et al. 2017b
84	*L. purpurus* (Yang, Zeng & Wang, 2018)	Yang et al. 2018
85	*L. purpuraventra* Wang, Li, Li, Chen & Wang, 2019	Wang et al. 2019
86	*L. pyrrhops* (Poyarkov, Rowley, Gogoleva, Vassilieva, Galoyan & Orlov, 2015)	Poyarkov et al. 2015
87	*L. rowleyae* (Nguyen, Poyarkov, Le, Vo, Ninh, Duong, Murphy & Sang, 2018)	Nguyen et al. 2018
88	*L. sabahmontana* (Matsui, Nishikawa & Yambun, 2014)	Matsui et al. 2014a
89	*L. serasanae* Dring, 1983	Dring 1983
90	*L. shangsiensis* Chen, Liao, Zhou & Mo, 2019	Chen et al. 2019
91	*L. shimentaina* Wang, Lyu & Wang, 2022	Wang et al. 2022
92	*L. sinorensis* Matsui, Panha & Eto, 2023	Matsui et al. 2023
93	*L. sola* (Matsui, 2006)	Matsui 2006
94	*L. suiyangensis* Luo, Xiao, Gao & Zhou, 2020	Luo et al. 2020
95	*L. sungi* (Lathrop, Murphy, Orlov & Ho, 1998)	Lathrop et al. 1998
96	*L. shiwandashanensis* Chen, Peng, Pan, Liao, Liu & Huang, 2021	Chen et al. 2021c
97	*L. tadungensis* (Rowley, Tran, Le, Dau, Peloso, Nguyen, Hoang, Nguyen & Ziegler, 2016)	Rowley et al. 2016
98	*L. tamdil* (Sengupta, Sailo, Lalremsanga, Das & Das, 2010)	Sengupta et al. 2010
99	*L. tengchongensis* (Yang, Wang, Chen & Rao, 2016)	Yang et al. 2016
100	*L. tuberosa* (Inger, Orlov & Darevsky, 1999)	Inger et al. 1999
101	*L. ventripunctata* (Fei, Ye & Li, 1990)	Fei et al. 1990; Fei et al. 2009, 2016
102	*L. verrucosa* Wang, Zeng, Lin & Li, 2022	Lin et al. 2022
103	*L. wuhuangmontis* Wang, Yang & Wang, 2018	Wang et al. 2018
104	*L. wulingensis* Qian, Xia, Cao, Xiao & Yang, 2020	Qian et al. 2020
105	*L. wumingensis* Chen, Peng, Li & Yu, 2023	Chen et al. 2023
106	*L. xishuiensis* Luo, Deng, Zhou, 2025	Luo et al. 2025
107	*L. yingjiangensis* (Yang, Zeng & Wang, 2018)	Yang et al. 2018
108	*L. yongshunensis* Huang, Wu, Jiang & Zhang, 2025	Huang et al. 2025
109	*L. yunkaiensis* Wang, Li, Lyu & Wang, 2018	Wang et al. 2018
110	*L. yeae* Shi, Hou, Song, Jiang & Wang, 2021	Shi et al. 2021
111	*L. yunyangensis* Luo, Deng & Zhou, 2022	Luo et al. 2022
112	*L. zhangyapingi* (Jiang, Yan, Suwannapoom, Chomdej & Che, 2013)	Jiang et al. 2013

Taxonomic account

Leptobrachella yachangensis sp. nov.

https://zoobank.org/87019B8E-76B9-4738-9BCD-1876D40C40F4

Fig. 3

Yachang Leaf Litter Toad (in English) / Ya Chang Zhang Tu Chan (雅长掌突蟾 in Chinese)

Type materials. ***Holotype*** ♂.NHMG250402, collected by Yun-Ming Mo and Zhong Huang on 15 April 2025 from Guangxi Yachang Orchid National Nature Reserve (24°44'57.24" N, 106°19'38.94" E; ca. 1400 m a.s.l.), Leye County, Baise City, Guangxi, China.

Paratypes. 6♂: NHMG250401,NHMG250409-250414, the same collection data as the holotype; NHMG250401, collected by Yun-Ming Mo and Zhong Huang on 15 April 2025 from Guangxi Yachang Orchid National Nature Reserve (24°45'6.85" N, 106°19'22.38" E; ca. 1250 m a.s.l.); NHMG250409-250414, collected by Yun-Ming Mo and Zhong Huang on 16 April 2025 from Guangxi Yachang Orchid National Nature Reserve (24°44'52.82"N, 106°20'2.06"E; ca. 1610 m a.s.l.).

Etymology. The specific epithet, *yachangensis*, is in reference to the type locality, Yachang Orchids National Nature Reserve.

Diagnosis. (1) Small body size [SVL 28.1–31.2 mm in seven adult males], (2) iris bicolored, upper half coppery orange and lower half grayish brown, (3) tympanum distinct, (4) supratympanic line black, (5) fingers without lateral fringes, (6) toes with narrow lateral

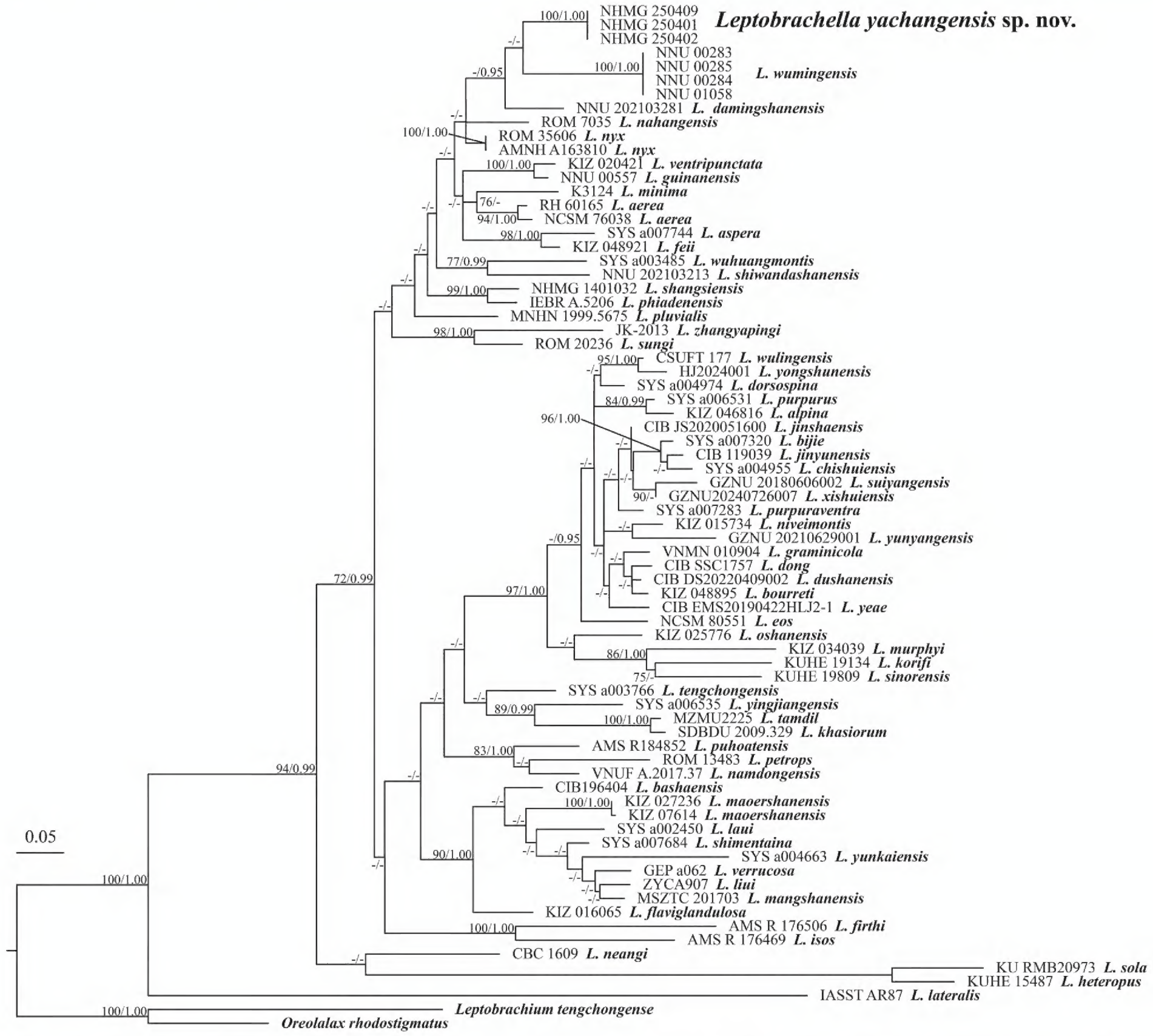

Figure 2. Maximum Likelihood tree results from partial DNA sequences of the mitochondrial 16S r RNA gene. Numbers before slashes indicate bootstrap support (BS) and numbers after slashes are Bayesian posterior probabilities (BPP). The '-' represent BS ≤ 70 or BPP ≤ 0.90. Voucher before species name at the branch terminal corresponds to the voucher ID in Suppl. material 1: table S1.

fringes,lacking webbing, (7) longitudinal ridges under toes continuous, (8) heels overlapping when adpressed, tibial-tarsal articulation reaching anterior corner of eye, (9) dorsal surface rough with dense skin ridges and numerous conical tubercles bearing spines, (10) ventral surface smooth, with transparent tiny conical spines on chest and abdomen in breeding males, (11) dorsum grayish brown, with darker brown scattered markings, (12) flanks with several dark spots, (13) surface of throat, chest and abdomen creamy white, with dense irregular dark brown speckles.

Description of holotype. Adult male. Body size small, SVL 31.2 mm. Head length slightly larger than head width, HDW/HDL 0.90; snout slightly protruding, projecting slightly beyond margin of lower jaw; nostril closer to snout than eye; canthus rostralis gently rounded; loreal region slightly concave; interorbital space flat, internarial distance slightly larger than interorbital distance, IND/IOD 1.07; pineal ocellus absent; pupil vertical; snout slightly longer than eye diameter, SNT/EYE 1.07; tympanum distinct, rounded, diameter smaller than that of eye and larger than tympanum-eye distance, TMP/EYE 0.48, TEY/TMP 0.48; upper margin of tympanum in contact with supratympanic ridge; vomerine teeth absent; a single vocal sac; vocal sac openings slit-like, paired, located posterolaterally on floor of mouth, close to margins of mandible; tongue deeply notched distally; supratympanic ridge distinct, extending from posterior corner of eye to supra-axillary gland.

Tips of fingers rounded, slightly swollen; relative finger lengths I < II < IV < III; nuptial pad absent; subarticular tubercles absent; inner palmar tubercle large, rounded, distinctly separated from outer palmar tubercle; outer palmar tubercle small, rounded; fingers lacking interdigital webbing and lateral fringes. Tips of toes

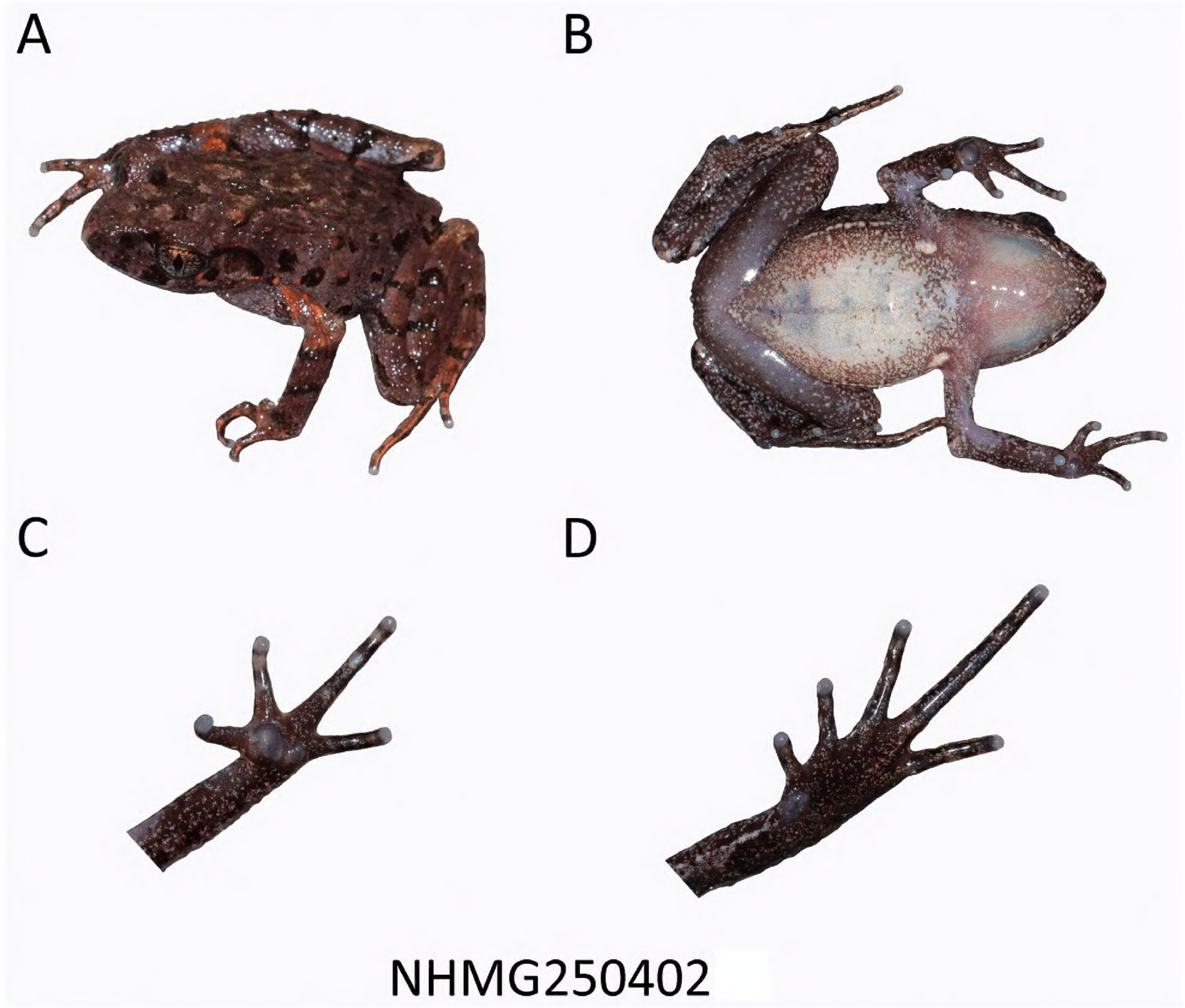

Figure 3. Morphological features of the holotype of *Leptobrachella yachangensis*sp. nov., NHMG250402 in life. **A.** Dorsal view; **B.** Dorsolateral view; **C.** Lateral view; **D.** Ventral view; **E.** Ventral view of hand; **F.** Ventral view of foot.

rounded, slightly swollen; relative toe length I < II < V< III < IV; longitudinal ridges under toes continuous; inner metatarsal tubercle large, oval; outer metatarsal tubercle absent; interdigital webbing between toes absent; narrow lateral fringes present on all toes. Tibia 47% of snout-vent length; tibiotarsal articulation reaches to anterior corner of eye; heels overlapping when thighs are appressed at right angles with respect to body.

Dorsal surface rough with dense skin ridges and numerous conical tubercles bearingspines; ventral skin smooth with transparent tiny conical spines on chest and abdomen; pectoral and femoral glands oval, both larger in diameter than tip of fingers; femoral glands larger in diameter than tip of toes; femoral gland situated on posteroventral surface of thigh, closer to knee than to vent; supra-axillary gland raised. Ventrolateral gland distinctly visible, forming a longitudinal uncontinuous series.

Coloration of holotype in life. Dorsal background color grayish brown, with darker brown markings. A dark brown inverted triangular marking in interorbital region, connecting to dark brown W-shaped marking on occipital region. Tympanum grayish brown. Supratympanic line black. Transverse dark brown bars on dorsal surfaces of distal limbs and digits; distinct dark brown spots on flanks; surfaces of digits, elbows, upper arms and tibiotarsal articulations coppery orange, with dark markings and spots.

Surface of throat, chest and abdomen creamy white, with dense irregular dark brown speckles. Underside of limbsgrayish brown with numerous irregular white speckles. Supra-axillary gland coppery orange, pectoral and femoral glands grayish white, ventrolateral glands grayish white. Iris bicolored, upper half coppery orange and lower half grayish brown.

Coloration of holotype in preservative. Dorsal background color dark brown scattered with irregular gray pigmentations. All markings, bars and spots become indistinct. Tympanum dark brown.

Ventral surface of throat, chest and abdomen grayish white; irregular dark brown speckles more distinct. Underside of limbs dark brown, irregular speckles grayish white. Supra-axillary, pectoral, and ventrolateral glands grayish white.

Variation. Measurements and body proportions are listed in Table 2. All the specimens showed relatively small differences in coloration and color patterns.

Distribution, ecology and conservation. *Leptobrachella yachangensis* sp. nov. is currently only known from its type locality (Fig. 1; ca. 1240–1620 m a.s.l.). The new species inhabits clear-water rocky streams in primary forests surrounded by broad-leaved forest in granite landforms of karst region. Males were observed calling while perched in the rock crevices or on the leaves of dwarf shrubs near flowing seeps. The breeding season lasts from February to June according to our long-term observation. During the breeding season, a large number of vocalizing individuals can be observed in the streams.*Leptobrachella yachangensisis* sp. nov.is under multiple potential human-induced pressures: potential wind power projects and eco-tourism development outside the protected area, artificial forest (such as Chinese fir forest) planting in the surrounding areas, and other activities may cause water pollution and habitat fragmentation. However, given that the current information on the distribution and threats of this species is still not fully understood, we recommend the new species be listed as Data Deficient (DD) according to the International Union for Conservation of Nature (IUCN) Red List Categories and Criteria.

Comparisons. In the phylogenetic tree (Fig. 2),*Leptobrachella yachangensis* sp. nov. is most closely related to *L. wumingensis* (mean *p*-distance 6.5%) and *L. damingshanensis* (mean *p*-distance 7.2%), therefore, these two species are selected for detailed comparison. The new species differs from *L. wumingensis* by its presence of narrow fringes along lateral surfaces of toes (vs. absence of lateral fringes on toes), and differs from *L. damingshanensis* by its absence of webbing on toes (vs. presence of webbing on toes). Moreover, the new species can be also distinguished from the later two species by its presence of numerous conical tubercles bearing spines on dorsal surface (vs. absence of spines), the presence of transparent tiny conical spines on chest and abdomen in breeding males (vs. absence of spines), presence of dense irregular dark brown speckles on throat, chest and abdomen with (vs. absence of such speckles).

Compared with the 26 known congeners of the genus *Leptobrachella* occurring south of the Isthmus of Kra, by the presence of supra-axillary and ventrolateral glands, *L. yachangensis* sp. nov. can easily be distinguished from *L. arayai*, *L. dringi*, *L. fritinniens*, *L. gracilis*, *L. hamidi*, *L. heteropus*, *L. kajangensis*, *L. kecil*, *L. marmorata*, *L. melanoleuca*, *L. maura*, *L. picta*, *L. platycephala*, *L. sabahmontana*, and *L. sola*, all of which lack supra-axillary and ventrolateral glands; and by its distinctly larger male body size, SVL 25.7–28.2 mm, *L. yachangensis* sp. nov. differs from the smaller *L. baluensis* (SVL 14.9–15.9 mm), *L. brevicrus* (SVL 17.1–17.8 mm), *L. bondangensis* (SVL 17.8 mm), *L. fusca* (SVL 16.3 mm), *L. itiokai* (SVL 15.2–16.7 mm), *L. juliandringi* (SVL 17.0–17.2 mm), *L. mjobergi* (SVL 15.7–19.0 mm), *L. natunae* (SVL 17.6 mm), *L. parva* (SVL 15.0–16.9 mm), and *L. palmata* (SVL 14.4–16.8 mm); and even distinctly larger than female *L. serasanae* (SVL 16.9 mm).

In having black spots on flanks, *Leptobrachella yachangensis* sp. nov. further differs from *L. aerea*, *L. botsfordi*, *L. crocea*, *L. eos*, *L. firthi*, *L. isos, L. pallida*, *L. petrops*, and *L. tuberosa*, all of which lack black spots on the flanks.

For the remaining 75 members of the genus *Leptobrachella*, males of the new species (SVL 28.1–31.2 mm) differ from males of the larger *L. nahangensis* (40.8 mm), *L. sungi* (48.3–52.7 mm), and *L. zhangyapingi* (48.5–52.5 mm); and males of the smaller *L. applebyi* (19.6–22.3 mm), *L. aspera* (22.4 mm), *L. feii* (21.5–22.8 mm), *L. melica* (19.5–22.7 mm), *L. niveimontis* (22.5–23.6 mm), *L. pluvialis* (21.3–22.3 mm), and even distinctly larger than female *L. korifi* (22.7 mm).

Through its toes without webbing, *Leptobrachella yachangensis* sp. nov. can be distinguished from *L. albomarginata*, *L. alpina*, *L. bashaensis*, *L. bijie*, *L. bidoupensis*, *L. bourreti*, *L. chishuiensis*, *L. dayaoshanensis*, *L. dong*, *L. dorsospina*, *L. dushanensis*, *L. guinanensis*, *L. fuliginosa*, *L. flaviglandulosa*, *L. graminicola*,

Table 2. Measurements (minimum–maximum (mean ± SD); in mm), and body proportions of*Leptobrachella yachangensis* sp. nov.

Vocher	NHMG 250401	NHMG 250402 (Holotype)	NHMG 250409	NHMG 250411	NHMG 250412	NHMG 250413	NHMG 250414	Range
Sex	Male	Male	Male	Male	Male	Male	Male	Males (n = 7)
SVL	28.3	31.2	30.0	28.7	28.9	28.1	29.8	28.1–31.2 (29.3 ± 1.1)
HDL	11.1	11.7	11.4	11.0	10.8	11.5	11.2	10.8–11.7 (11.2 ± 0.3)
HDW	10.1	10.5	10.1	10.0	10.4	10.3	11.6	10.0–11.6 (10.4 ± 0.5)
SNT	4.4	4.7	4.5	4.4	4.4	4.6	4.5	4.4–4.7 (4.5 ± 0.1)
IND	2.8	3.1	3.0	2.9	3.1	2.9	3.1	2.8–3.1 (3.0 ± 0.1)
IOD	2.7	2.9	2.8	2.7	2.8	2.6	2.8	2.6–2.9 (2.8 ± 0.1)
EYE	4.2	4.4	4.1	4.1	4.2	4.3	4.5	4.1–4.5 (4.3 ± 0.2)
TMP	1.9	2.1	2.0	2.1	2.0	2.1	2.1	1.9–2.1 (2.0 ± 0.1)
TEY	1.0	0.7	0..8	0.8	0.9	0.8	0.9	0.7–1.0 (0.9 ± 0.1)
ML	8.6	8.2	8.3	8.5	8.2	8.3	8.4	8.2–8.6 (8.4 ± 0.2)
LAHL	16.2	15.4	15.7	16.0	15.5	15.8	15.9	15.4–16.2 (15.8 ± 0.3)
PL	14.7	13.7	14.3	14.6	14.2	13.8	14.4	13.7–14.7 (14.2 ± 0.4)
TIB	14.3	14.7	14.1	14.9	14.5	14.2	14.8	14.1–14.9 (14.5 ± 0.3)
HLL	42.7	46.8	44.6	46.3	45.7	44.4	45.9	42.7–46.8 (45.2 ± 1.4)

L. jinyunensis, *L. khasiorum*, *L. lateralis*, *L. laui*, *L. liui*, *L. macrops*, *L. mangshanensis*, *L. maoershanensis*, *L. minima*, *L. murphyi*, *L. namdongensis*, *L. neangi*, *L. nokrekensis*, *L. nyx*, *L. pelodytoides*, *L. puhoatensis*, *L. purpurus*, *L. purpuraventra*, *L. pyrrhops*, *L. phiadenensis*, *L. phiaoacensis*, *L. pingbianensis*, *L. rowleyae*, *L. shangsiensis*, *L. shimentaina*, *L. sinorensis*, *L. suiyangensis*, *L. tamdil*, *L. tengchongensis*, *L. ventripunctata*, *L. verrucosa*, *L. wulingensis*, *L. xishuiensis*, *L. yongshunensis*, *L. yingjiangensis*, *L. yunkaiensis*, *L. yunyangensis*, and *L. yeae*, all of which bear webbing on toes. In having toes with narrow lateral fringes, the new species can be distinguished from *L. ardens*, *L. kalonensis*, *L. maculosa*, *L. oshanensis*, *L. shiwandashanensis*, and *L. tadungensis* (vs. lateral fringes absent). In having transparent tiny conical spines on chest and abdomen in breeding males, the new species can be distinguished from *L. aurantirosea*, *L. batxatensis*, *L. huynhi*, and *L. jinshaensis* (vs. ventral skin smooth).

Discussion

The rapid discovery of numerous cryptic species within the genus *Leptobrachella* across southern China in the past five years (37 new species described since 2020, 10 of which are from Guangxi; Chen et al. 2020; Wang et al. 2022; Liu et al. 2023; Li et al. 2024) strongly indicates that the true diversity of this genus remains severely underestimated. Our discovery of the sympatric occurrence of *Leptobrachella yachangensis* and *L. ventripunctata* in rocky stream habitats within the karst mountains of Leye County, Guangxi (bordering Guizhou), further corroborates the findings of Rowley et al. (2015): highly divergent lineages within *Leptobrachella* frequently exhibit pronounced micro-endemism and sympatry. This complex distribution pattern significantly complicates species delimitation and classification within the genus. Furthermore, the widespread morphological conservatism among *Leptobrachella* species (Wang et al. 2019; Chen et al. 2020), resulting in highly similar appearances of many congeners in the field, not only hinders research into their diversification mechanisms but also poses challenges for developing targeted conservation strategies.

Molecular phylogenetic analysis (Fig. 2) revealed that *L. yachangensis* is sister to *L. wumingensis*, not to the sympatrically occurring *L. ventripunctata*. This phenomenon of "closely related species being allopatric, while distantly related species co-occurring sympatrically" is not unique within *Leptobrachella*. For instance: in Guangdong Shimentai National Nature Reserve, *L. shimentaina* co-occurs with the phylogenetically distant *L. mangshanensis* (Wang et al. 2022); in Yingjiang County of Yunnan, *L. purpurus* is found sympatrically with the phylogenetically distant *L. yingjiangensis* and *L. ventripunctata*(Yang et al. 2018). These cases collectively demonstrate a prevalent pattern of "decoupling of phylogeny and sympatry" within *Leptobrachella*, suggesting that niche differentiation (e.g., microhabitat preference, breeding season isolation) or historical dispersal events may play a more critical role than shared ancestry in driving sympatric distribution patterns (Rowley et al. 2015). Geographically, the only currently known locality of *L. yachangensis* (Yachang Orchid NNR, Leye, Guangxi) is approximately 250 km in a straight line from the type locality of its sister species, *L. wumingensis* (Mt Damingshan, Wuming, Guangxi), separated by the Hongshui River valley and continuous non-karst mountains. This moderate-scale geographic isolation, combined with significant genetic divergence (*p*-distance 4.1–4.9%), supports the hypothesis of karst landscapes acting as "evolutionary islands" (Ficetola et al. 2021), where isolated habitat patches drive the independent evolution of genetic lineages.

Leptobrachella yachangensis is currently known only from primary montane forests at elevations of 1240–1620 m within the Yachang Orchid National Nature Reserve, Leye County, Guangxi. Its survival is under multiple potential human-induced pressures: potential wind power projects and eco-tourism development outside the protected area, artificial forest (such as Chinese fir forest) planting in the surrounding areas, and other activities may cause water pollution and habitat fragmentation. Climate change may also affect the micro-environment of the alpine streams it depends on. Therefore, we recommend the immediate implementation of the following conservation measures: 1) Expand survey efforts: Systematically survey adjacent karst regions (e.g., Maolan NNR in Guizhou, Mulun NNR in Guangxi) to assess the species' actual distribution range and population connectivity; 2) Strengthen habitat protection: Strictly limit anthropogenic disturbances (e.g., trail construction, tourist access) within core stream habitats in the reserve and maintain the integrity of the primary forest vegetation; 3) Establish dynamic monitoring: Implement a population and habitat monitoring network to evaluate the long-term impacts of tourism and quarrying activities. Given its extremely limited distribution and evident threats, and although current data is insufficient for a precise assessment, we strongly recommend its inclusion in the China Biodiversity Red List and prioritization for an IUCN Red List assessment in the near future (adopting the precautionary principle from DD towards potentially VU).

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Supplementary material 1

Collection localities, voucher data, and Genbank accession numbers (16S rRNA) for all *Leptobrachella* samples used in this study.

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Data type: docx

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